Perfecting the ring and extending the antibacterial spectrum: 'the multiple generations'

A. Bryskier

Clinical Pharmacology of Anti-infectives, Hoechst Marion Roussel, Romainvillle, France

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Sulfonamides, tyrothrycin and penicillin G were the forerunners in an intensive successful research to discover new antibacterial agents. Many teams were looking for new compounds, especially those with a broad antibacterial spectrum.

The success of penicillin G had an unexpected effect: an increasing number of *Staphylococcus aureus* isolates resistant to penicillin G in London hospitals spread progressively all round the world. Research into novel antibacterial agents was extremely important. Many compounds with antibacterial activity were discovered and screened against *S. aureus* isolates resistant to penicillin G. Erythromycin, vancomycin, methicillin and oxacillin were discovered or synthesized in the 1950s.

In Sardinia, in 1945, G. Brotzu discovered that fermentation products extracted from *Cephalosporium acremonium* isolated from the sea near a sewage outfall at Cagliari, Sardinia exhibited anti-Gram-positive activities. The fermentation extracts were successfully administered locally for the treatment of wound infections [1]. As Brotzu was unable to purify the *C. acremonium* extracts, he contacted the Oxford team who had been successful with penicillin to extract and purify the components of this fermentation.

Newton and Abraham discovered cephalosporin C in 1955 [2]. Cephalosporin C was found to have several remarkable properties, being active against *Escherichia. coli, Salmonella typhi* and the Oxford strain of *S. aureus*, but also resistant to hydrolysis by penicillinase from *Bacillus subtilis*. The chemical structure was elucidated in 1959 [3]. In 1960, small amounts of 7-amino-cephalosporanic acid (7-ACA) were obtained by acid hydrolysis of cephalosporin C (Figure 1).

A chemical procedure by which the α-aminoadipyl side-chain could be removed to produce 7-ACA in good yield was developed in 1962 [4]. Four classifications have been described: biological [5], microbiological [6], chemical [7,8] and pharmacokinetic [6].

Chemical classification is made according to ring alterations or by substituent alterations (Figures 2 and 3).

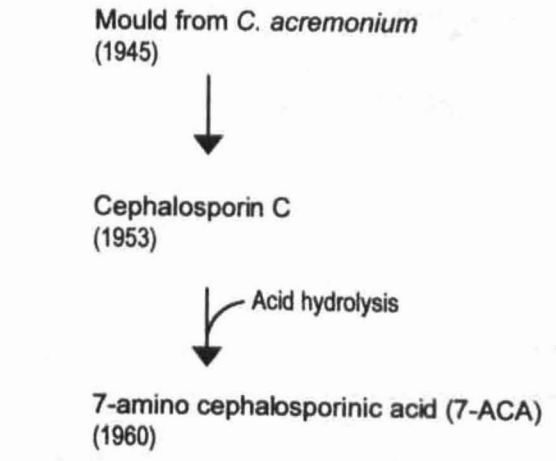


Figure 1 Discovery of cephalosporin C.

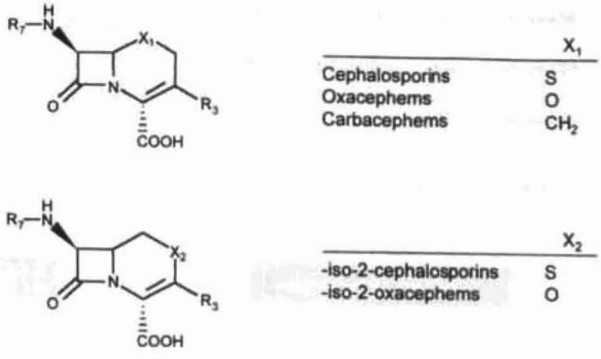


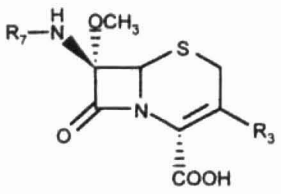
Figure 2 Cephems: classification 2, chemical classification 1, modification of the ring.

Six waves of cephalosporins enable (Figure 4) a microbiological classification to be made, in which cephems are divided into seven groups (Figure 5) according to the antibacterial spectrum.

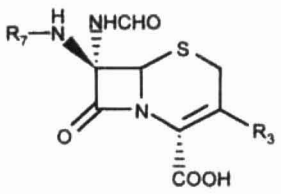
The development of numerous compounds, which were introduced in clinical practice, allowed cephems to be divided into three pharmacokinetic groups according to their apparent elimination half-life (Figure 6).

In 1964, cephalothin and cephaloridine were introduced in clinical practice, followed by cefazolin. Cephaloridine was the first cephem with a C-3 azolium heterocycle substituent.

Corresponding author and reprint requests: A. Bryskier, Clinical Pharmacology of Anti-infectives, Hoechst Marion Roussel, 102, route de Noisy, 93235 Romainvillle, France
Fax: +33 1 49 97 50 20

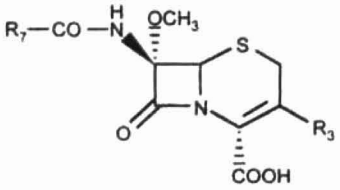


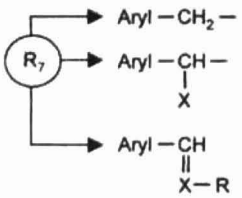
Cephamycins



Cephabicins

Figure 3 Cephems: classification 3, chemical classification 2, modification of substituents.





Wave 1	*S. aureus* penicillin G-resistant	Cephalothin cephaloridine
Wave 2	Gram-negative bacilli	Cefuroxime Cefamandole Cefoxitin
Wave 3	Gram-negative bacilli (TEM-1/SHV-1)	Cefotaxime Ceftriaxone
	P. aeruginosa	Ceftazidime Cefsulodin
Wave 4	Gram-negative bacilli (Case)	Cefpirome Cefepime
Wave 5	Gram-negative bacilli (ESBL)	Ceftecol RU 59863
Wave 6	MRSA	MC-02,331 LY 274,858

Figure 4 Successive waves of cephems.

Many compounds were synthesized and these composed group I of the microbiological classification. These analogs were prepared to overcome *S. aureus* resistance to penicillin G. The second aim was to prepare compounds with a new C-3 side chain to avoid metabolism and to lengthen the apparent elimination half-life. One compound, cefazolin, possesses a long apparent half-life.

The successful introduction of ampicillin in clinical practice, which exhibits anti-Gram-negative activity, was rapidly followed by the emergence of clinical isolates resistant to ampicillin by the production of broad spectrum β-lactamases.

Research for new cephem derivatives designed to overcome β-lactamase production in Gram-negative bacteria resulted in group II of the cephems.

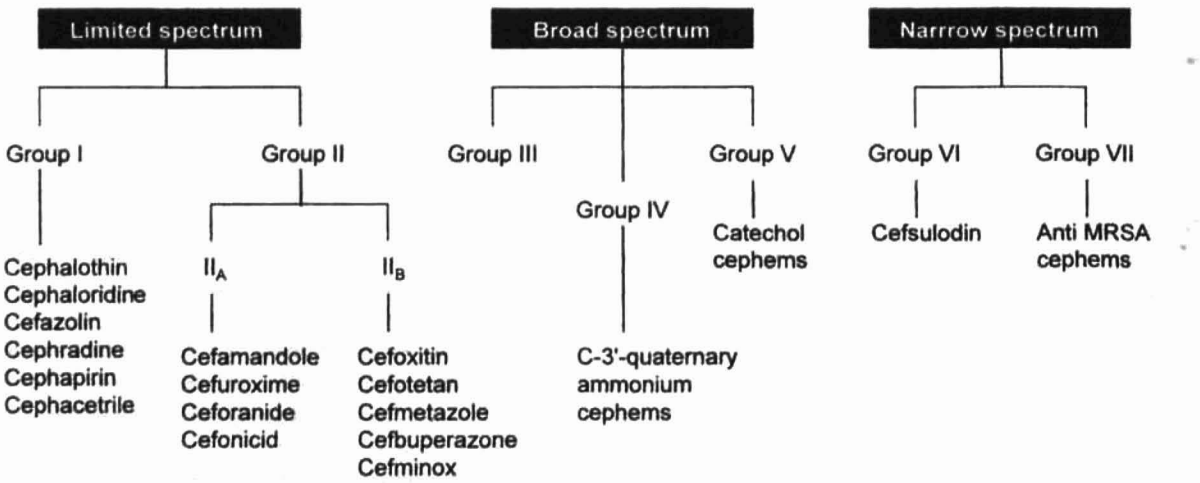


Figure 5 Cephems: microbiological classification.

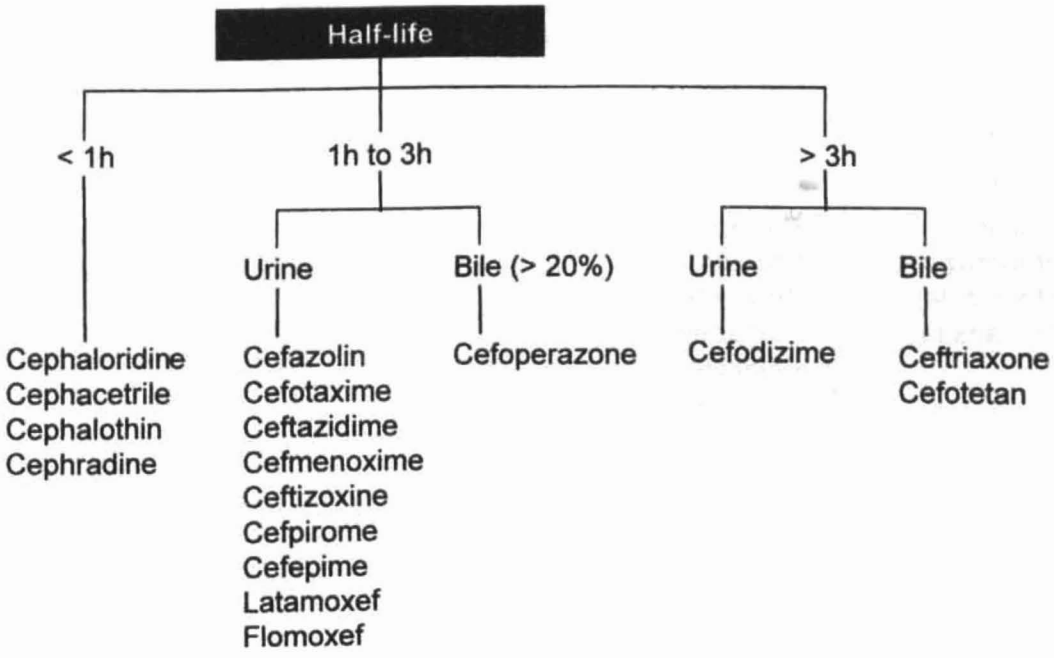


Figure 6 Cephems: pharmacokinetics classification.

At the beginning of the 1970s reports appeared that certain species of *Streptomyces* produced 7-α-methoxy cephalosporins [6], named cephamycins. The presence of a 7-α-methoxy group in the cephem ring was found to be associated with high resistance to hydrolysis by most β-lactamases. During the 1970s several new cephalosporins with useful activity against Gram-negative bacilli were prepared by further alterations of the C-3 and C-7 side-chains.

Two important discoveries in the field of cephems were reported: the *syn* methoxyimino residue at the C-7 position of the cephem ring (as in cefuroxime) and the thiomethyltetrazole ring (as in cefamandole), which leads to enhancement of in vitro activity. These compounds of group II were introduced in clinical practice in 1979–80. In general, they are less active than group I compounds against staphylococci and streptococci but are more active against selected Gram-negative bacilli. All these cephems possess an apparent elimination half-life range from 1 to 3 h. One compound, cefonicid, with a modification on the C-3 tetrazolyl ring, having a sulfonyl group instead of a methyl group, possesses a long apparent elimination half-life of 5 h [6].

A continuous flow of new compounds issued from chemical alterations conducted to design new derivatives to overcome β-lactamases hydrolysis, allowed cefotaxime, the first compound of group III, to be developed [9,10].

In parallel, two types of compounds have been synthesized: N-acyl cephalosporins represented by cefoperazone, or 2-amino-5-thiazolyl cephalosporins represented by cefotaxime, all of them are included in group III. A given cephem can be assigned to group III, if it possesses at least two of the following characteristics: the chemical structure includes a 2-amino-5-thiazolyl ring; stability to broad-spectrum α-lactamase hydrolysis; good activity against Gram-negative bacilli, e.g. Enterobacteriaceae, or fastidious Gram-negative bacilli; antipseudomonal activity (Figure 7).

Cefotaxime was the first 2-amino-5-thiazolyl cephem having a *syn* methoxyimino residue at C-7. The C-3 chain is an acetoxymethyl moiety. The *syn*-methoxyimino group is responsible for the high stability to hydrolysis by broad-spectrum β-lactamases.

The introduction of the 2-amino-5-thiazolyl ring in the C-7 side chain is a major advance in cephalosporin research. This ring is responsible for the breakthrough in antibacterial activity, as cefotaxime is more than 100 times more potent than cefamandole against Gram-negative bacteria (Figure 8).

Numerous other 2-amino-5-thiazolyl cephems were synthesized and introduced in clinical practice. They differ from cefotaxime in their substituents at position 3 of the cephem ring. At least five compounds in addition to cefotaxime were introduced in clinical practice: ceftizoxime, ceftriaxone, cefodizime, ceftazidine, cefmenoxime (Figure 9).

The C-3 substituent is responsible for pharmacokinetic properties, highlighted in ceftriaxone, which possesses a triazine substituted ring, or cefodizime, which has a 3′,5′-disubstituted thiazolyl ring [11] (Figure 10). The latter substituent is also responsible for 'nonantibacterial activity',

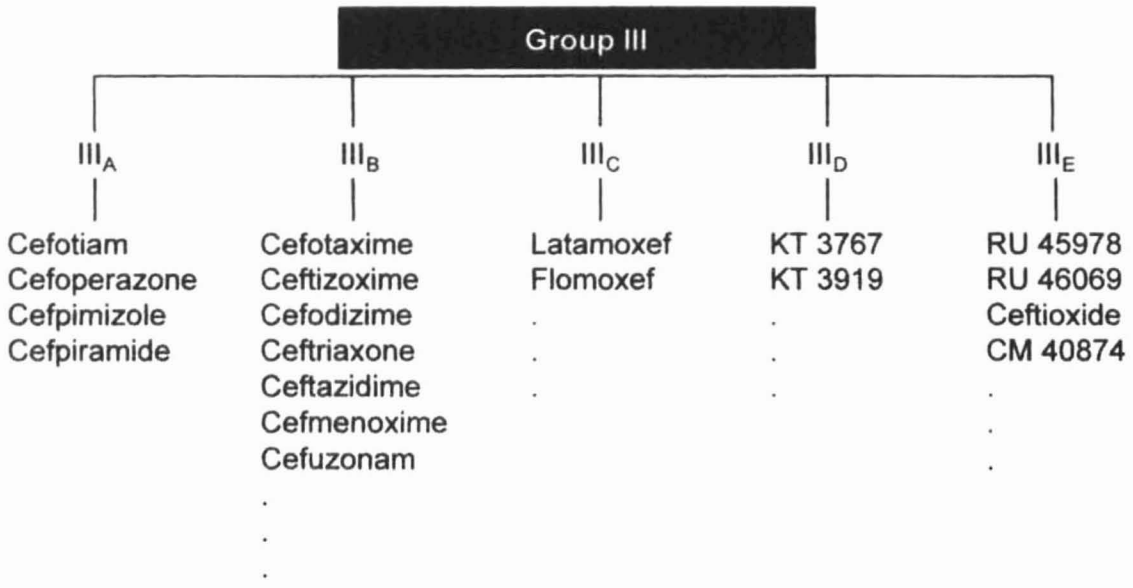


Figure 7 Cephems: classification: five subgroups A–E.

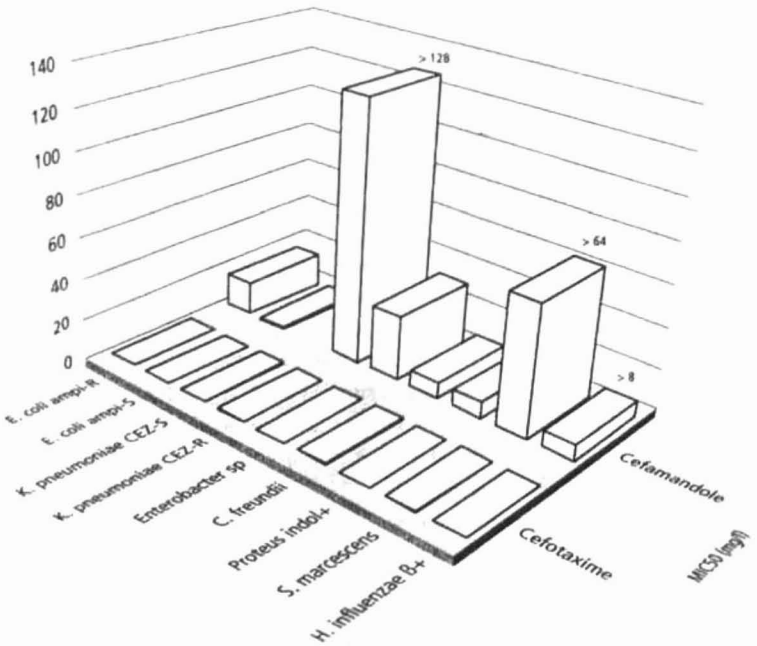


Figure 8 Cephems group III.

such as the immune-stimulating properties of cefodizime [12]. The C-3 moiety also contributes to antibacterial activity. This fact is highlighted by the inoculum size effect [13] or by differential activity against *Streptococcus pneumoniae* isolates resistant to penicillin G (Tables 1 and 2). Against such multidrug resistant *S. pneumoniae* strains, only cefotaxime and ceftriaxone exhibit good and therapeutic activity. Ceftizoxime, ceftazidime and cefodizime are devoid of antibacterial activity against these isolates [14] (Figure 11).

A third line of compounds was obtained by introduction of an oxygen atom in the cephem ring instead of a sulfur atom [15] (Figure 12). The first derivative introduced in clinical practice was latamoxef [15]. Latamoxef is active against Enterobacteriaceae harboring broad spectrum β-lactamases and even extended spectrum β-lactamases (ESBLs). However, latamoxef has low activity or is inactive against Gram-positive cocci. Latamoxef was given up due to the unexpected adverse events. The presence of an N-methyltetrazolthio ring at C-3′

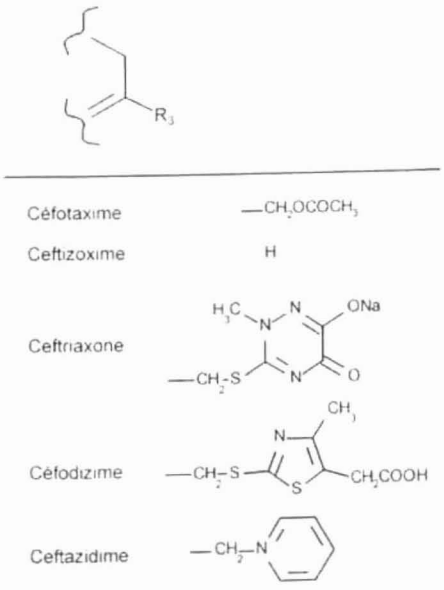


Figure 9 Cephem group III.

Table 1 Inoculum size effect *in vitro* (MIC: mg/L) [13]

	E. coli		*K. pneumoniae*	
CFU/mL	5×10^5	5×10^7	5×10^5	5×10^7
Cefotaxime	0.06	0.5	0.06	0.5
Ceftriaxone	0.06	1.0	0.25	4.0
Ceftazidime	0.12	8.0	0.12	8.0

has been shown to be responsible for hypoprothrombinaemia (inhibition of vitamin K-epoxide reductase) and antabuse-like effect (inhibition of aldehyde dehydrogenase). The carboxyl group at C-7 can cause platelet dysfunction [16].

However, the C-3′-N-hydroxymethyltetrazolylthiomethyl group of flomoxef does not inhibit the aldehyde dehydrogenase

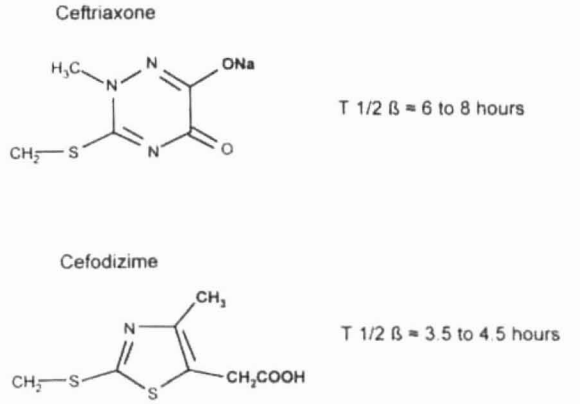


Figure 10 Group III B cephems with long apparent elimination half-life.

[17] and is less inhibiting toward vitamin K-epoxide reductase than the N-methyl derivative. Flomoxef is less active than latamoxef against Enterobacteriaceae producing class 1 β-lactamases but shows a good in vitro activity against Gram-positive cocci. Other oxa-1-cephems have been synthesized but none of them reached the development stage [6].

Today, three cephalosporins of group III remain major antibacterial drugs for treatment of severe infections: cefotaxime, ceftriaxone and ceftazidime. A revival of ceftizoxime is expected in Japan as an oral aminoacid prodrug, but due to the lack of activity against *S. pneumoniae* isolates resistant to penicillin G, this compound will have restricted clinical indication in respiratory tract infections.

Bacteria are very flexible and always found a way to escape antibacterial activity. Group III compounds have weaknesses, they are devoid of activity against isolates producing class C β-lactamases (e.g. *Citrobacter freundii*, *Morganella morganii*, *Serratia marcescens*, *Enterobacter* spp.) and are hydrolyzed by ESBLs such as TEM-3 and other TEM enzymes, and SHV-2 and other SHV enzymes, which hydrolyze cefotaxime and ceftazidime in various degrees. ESBL enzymes are encountered mainly in *Klebsiella pneumoniae* but are also found in other *Enterobacter-*

Table 2 Activities of cephems against *S. pneumoniae* with different patterns of resistance to penicillin G [14]

	Peni-S (MIC < 0.12 mg/L)		Peni-I (MIC 0.12 ≤ 1.0 mg/L)		Peni-R (MIC > 1.0 mg/L)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Cefotaxime	0.03	0.03	0.25	1.0	1.0	2.0
Ceftriaxone	0.03	0.03	0.25	1.0	1.0	2.0
Ceftazidime	0.25	0.25	2.0	16.0	16.0	16.0
Ceftizoxime	0.06	0.06	0.5	8.0	16.0	16.0
Cefodizime	0.03	0.03	0.5	2.0	2.0	4.0
Cefpirome	0.01	0.03	0.12	0.5	0.5	1.0
Cefepime	0.03	0.12	0.12	0.5	1.0	1.0

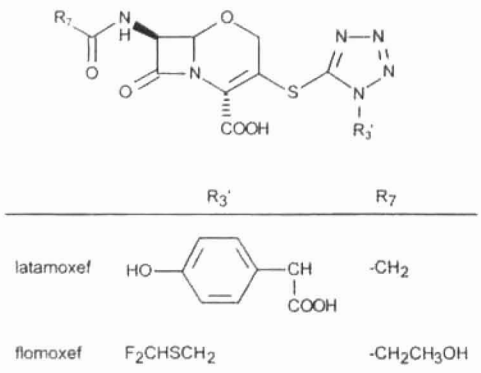


Figure 11 Cephems: group III. Oxa-1-cephems.

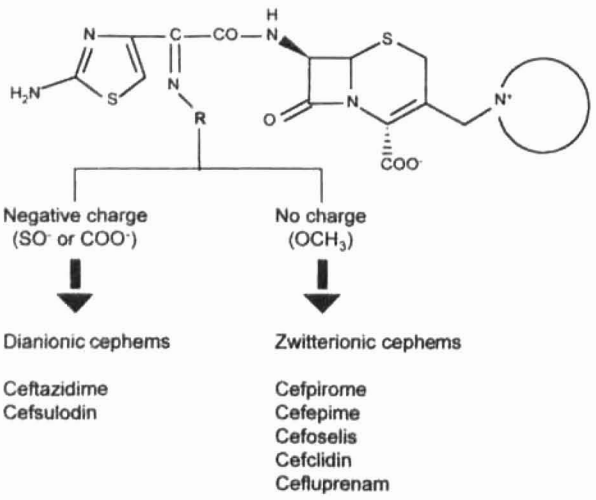


Figure 12 Cephems zwitterionic compounds.

iaceae species. The third weak point (except for ceftazidime) is the poor antipseudomonal activity.

Research did not stop after the introduction in clinical practice of members of group III. The target was to enhance antipseudomonal, antistaphylococcal activities and to overcome class C β-lactamases.

Cefsulodin [18] and ceftazidime were the first cephalosporin with improved activity *Pseudomonas aeruginosa* to be used in clinical setting. They bear a 1-pyridinium group at the C-3 position. These two compounds are dianionic compounds [19] (Figure 13). In the meantime, it was decided to abandon the use of the N-methyltetrazolylthio moiety at C3 due to unacceptable side-effects. Group IV, containing C-3′ quaternary ammonium cephem [20,21], arose from this research (Figure 14). These azolium derivatives are zwitterionic compounds (Figure 15). In group IV, other chemical alterations have been done, in order to increase antipseudomonal activity. Introduction of the 5-amino-2-thiadiazolyl ring instead of the 2-amino-5-thiazolyl ring (i.e. cefclidin, ceftuprenam) enhances the antipseudomonal activity but leads to decreased activity against Enterobacteriaceae [22] (Figure 16). Cefpirome, cefepime, cefclidin and ceftuprenam have been introduced in clinical practice.

Because of their zwitterionic nature, these compounds can enter through additional porin channels and rapidly reach their PBPs targets by exhibiting poor affinity for C class β-lactamases, which are located in periplasmic space. However, these compounds are hydrolyzed by ESBL and none of them show an elimination half-life above 3 h in healthy volunteers.

In order to increase anti-Gram-positive activities, different chemical modifications have been proposed, e.g. a C-3 vinyl moiety or an hydroxyimino chain instead of a methoxyimino residue at C-7 of the cephem ring [6]. These compounds showed a significant enhancement of antistaphylococcal activity. Introduction of a monofluoromethoxyimino group at the 7-position (ceftuprenam) led to a twofold increase in

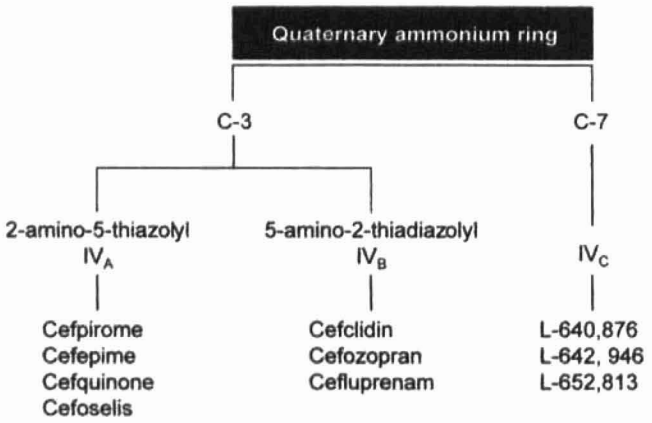


Figure 13 Cephems: group IV (2) classification.

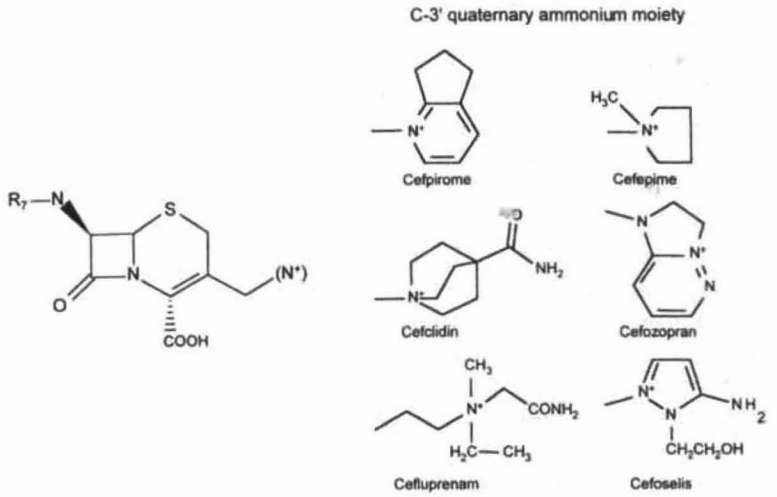


Figure 14 Group IV C-3′ ammonium quaternary cephems

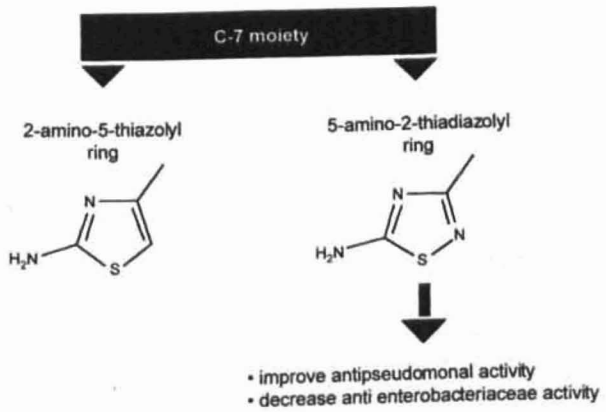


Figure 15 Cephems: group IV: chemical alteration

activity against most bacteria as compared with the methoxyimino counterpart [6]. However, the antipseudomonal activity is never satisfactory, as *P. aeruginosa* quickly overcomes the antibacterial activity of new compounds.

Cephems that belong to group IV possess two or more of the following characteristics:

1 the presence of a C-3′ quaternary ammonium side chain;
2 a broad antibacterial spectrum including *P. aeruginosa*;
3 good antibacterial activity against Enterobacteriaceae isolates producing class C enzymes.

They also have to fulfil the definition of group III [6].

All the C-3′ quaternary ammonium compounds introduced for clinical use or under development possess the same antibacterial activity as cefotaxime, but they differ by adding antipseudomonal activity [23]. Due to the C-3′ side-chain, there are differences among these compounds. Against *S. pneumoniae*, irrespective of the resistance phenotype to penicillin G, the most active compound is cefpirome. Against penicillin G resistant *S. pneumoniae* cefepime and cefozopran exhibit comparable activity to cefotaxime. Ceftuprenam was weakly active and cefclidin is inactive [21].

New cephems were needed against Enterobacteriaceae producing ESBL and *P. aeruginosa*. New sets of cephalosporins, which composed group V (Figure 17), are catechol or pyridone-containing cephems (Figure 18). These catechol or pyridone moieties are attached to the oxime residue, and produce a bifunctional mechanism of action, PBPs inhibition and iron chelation. None of them reached the registration

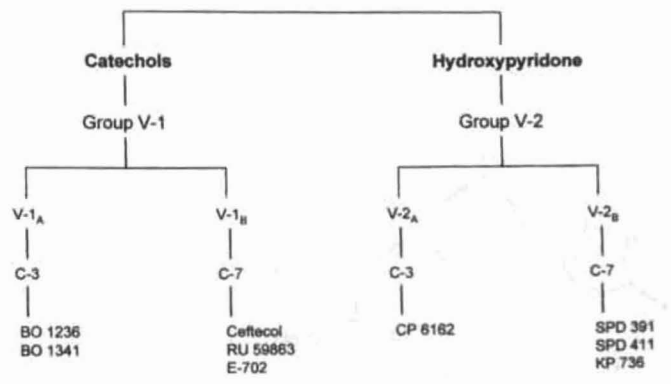


Figure 16 Cephems: group V (2): classification

stage. The furthest developed compound was ceftecol [24]. Some derivatives, such as RU59863, exhibited good anti-pseudomonal activity, were able to overcome ESBL production and displayed good antistaphylococcal activity [25,26].

Research into catechol-containing cephems was given up due to the difficult chemistry, high cost of production and risks of adverse events, as shown with ceftecol.

Today research in the cephem field is more focused on narrow spectrum antibacterials. The first narrow spectrum cephalosporin, cefsulodin (group VI), was introduced in clinical practice in the early 1980s. A renewal of interest on narrow antibacterial spectrum cephems arose in respect of methicillin-resistant *S. aureus* (MRSA) [27]. Many compounds have been investigated for anti-MRSA activities, within known families, such as glycopeptides, or new chemical entities for human use, such as oxazolidinones, everninomycin or new screened compounds.

By chemical alterations of the C-7 and C-3 substituents of the cephem nucleus, novel entities have been synthesized within carbacephems [28] or cephalosporins (Figure 18). The same type of research was ongoing in the carbapenem family [29,30]. This group VII is composed of narrow spectrum cephems. One compound is currently under development – MC 02479 (RWJ 54428). The difficult task is to produce cephems, which provide activity against MRSA and against GISA strains. RWJ 54428 [31] seems to be active against both MRSA and vancomycin-resistant *S. aureus* (GISA) strains. One known weakness of this compound is the poor water solubility. Prodrug derivatives have been prepared with improved solubility [32].

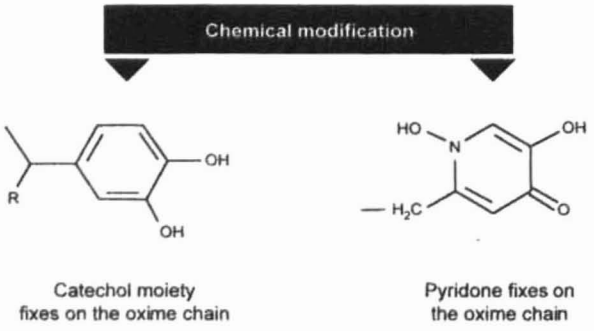


Figure 17 Cephems: group V.

CONCLUSION

Since the first biological classification published by O'Callaghan [5] in 1975, many extensive reviews in the field of cephalosporins have been published [6–8,27]. Classifications evolved following the synthesis of new chemical structure with novel antibacterial activities.

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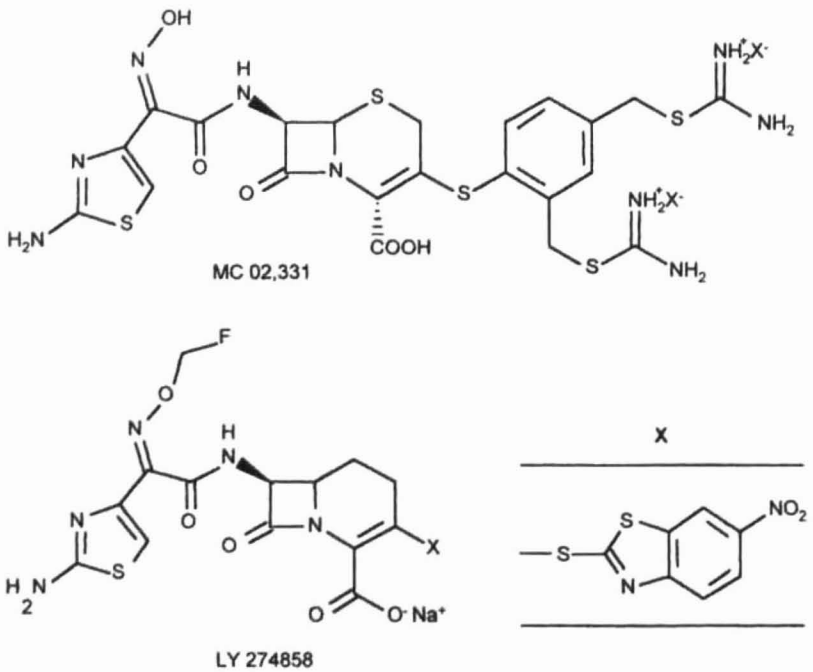

Figure 18 Cephems: group VII: cephems designed for anti-MRSA activity.

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